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ORIGINAL ARTICLE

Association Between Insulin Resistance and eNOS Activity in Type 2 Diabetic Iraqi Patients with Nephropathy

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ABSTRACT

Objectives: The current study aimed to evaluate the correlation between serum eNOS activity and other clinical characteristics in Iraqi DN subjects.

Methods: A case control study was done on 130 subjects, including 50 with DN, 40 with Type 2 Diabetes Mellitus (T2DM) without DN and 40 of them were healthy control. Phenotypic data, including serum eNOS activity, lipid profile, Kidney function tests, blood glucose, insulin, HbA1c and HOMA-IR were investigated.

Results: The current study shows there is non-significant difference in age, gender and BMI between DN, DM and HC groups (P- value >0.05 for both). A significant negative correlation was found between serum eNOS activity with creatinine, HOMA-IR among DN group, similar correlation between serum eNOS activity and Urea, HOMA-IR among DM group.

Conclusion: We found non-significant difference in age, gender and BMI between DN, DM and HC group. A significant negative correlation was found between serum eNOS activity, Creatinine and HOMA-IR among T2DM with DN group.

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INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder defined by high blood glucose level, inflammation, and oxidative stress [1].

During the last few decades, DM has become one of the greatest urgent and prevalent problems, with the increasing obesity ranked the seventh cause of death worldwide; unfortunately there is a 5.2 million diabetes global deaths with mortality rate of 82.4 per 100,000 [2].

There are many serious diabetes complications, including microvascular complications.[3] Diabetic nephropathy is the principal problem that could result in renal failure and end stage renal disease.[4] Diabetic nephropathy (DN) prevalence has increased across the globe.[5] DN is a specific indicator of both insulin-dependent and insulin-independent diabetes mortality.[6] Hence, prompt detection of diabetes and microvascular complication risk offers a chance to implement preventive interventions to prevent or postpone the onset of disease,[7] which is the most effective approach to reduce the morbidity and mortality of microvascular complications of T2DM patients.

Nitric oxide (NO) is a significant endogenous endothelial derived relaxing factor produced by the family of nitric oxide synthases (NOS family). Three mammalian NOS isoforms, most notably eNOS, are known to synthesize NO.[8] NO is produced by an enzymatic reaction and triggered by endothelial nitric oxide synthase (eNOS) from L-arginine.[9]

The most significant regulator of vascular homeostasis is nitric oxide (NO) and, thus, it's decreased bioavailability in the vasculature is a major characteristic of endothelial dysfunction.[10] Endothelial dysfunction (ED) plays a significant role in the development of multiple pathological conditions that lead to cardiovascular disease, such as hypercholesterolemia, hypertension, type 2 diabetes and chronic renal failure, ED identified as the impaired capacity of vascular endothelium to induce vasodilation.[11]

The characteristic feature of type 2 diabetes is insulin resistance and a broader clinical range such as glucose intolerance, obesity and metabolic syndrome can be established. The advancement of insulin resistance contributes to dyslipidemia (abnormal lipid metabolism). The consequences of insulin resistance and hyperinsulinemia are thought to derive from variations in the eNOS gene. The relationship between eNOS serum activity and HOMA-IR is positive and has been documented in several recent human studies.[8]

The aim of this study was to find the relation between the activity of eNOS with insulin resistance and other parameters in Iraqi DN patients.

MATERIALS AND METHODS

The study is a case control study, which carried out in the Al-Hassan Center for Diabetes & Endocrinology at Al-Hussein Medical Hospital, Kerbala Health Directorates, Kerbala, Iraq over the period. The patients had interviewed and a designed questionnaire has been filled. Blood sample (5 ml) was taken from the patients, the serum used for different biochemical laboratory investigations, including Kidney function tests, lipid profile, HbA1c, Insulin, blood glucose and eNOS activity. The eNOS activity was measured by ELISA, the insulin test was done through automatic calculation by utilizing the ARCHITECT PLUS i 1000 SR, while the Roche COBAS c311 used to evaluate the of rest biomarkers.

The total number of participants was 130 subjects which were divided into three groups: 50 of them had diabetic nephropathy (DN), 40 of them had Type two diabetes mellitus(T2DM) and 40 of them were healthy control (HC), whom they had been randomly chosen.

Averaged data are presented as the means ± SD. Pearson's correlation test was done in order to test any correlation among the values of the above parameters in patients with DN. As regards a P value, reading < 0.05 agreed to be statistically significant.

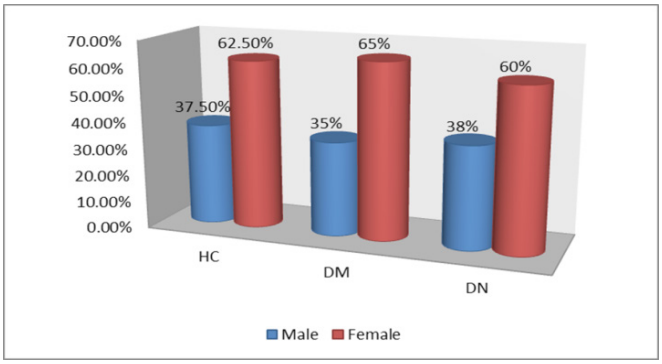

Figure 1. **Gender distribution of the studied individuals.**

RESULTS

One handred and thirty (130) subjects were recruited in this study, 50 patients with DN, 40 with T2DM without DN and 30 subjects were HC.

Gender Distribution

According to gender, the distribution of the DN group were 38% male and 60% female as shown in Figure 1. While the distribution of T2DM group were 35% male and 65% female, and the distribution of HC group were 37.5% male and 62.5% female.

Age Distribution

Patient's distribution according to age groups were 6% (38-47 years), 40% (48-57 years), 36% (58-67years) and 18% (68-77 years) was shown in Figure 2.

Distribution according to BMI

The patients distribution according to BMI groups were 6% (Underweight <18), 12% (Healthy 18.5-24.9), 40% (overweight 25-29.9) and 42% (Obese ≥ 30.0) shown in Figure 3.

Association between eNOS and different biochemical markers

In the present study, the relationship between (Urea, Creatinine, eGFR, FBS, HbA1c,TC, HDL-C, LDL-C, TG, HOMA-IR, Insulin, HOMA-B, S% and eNOS activity) was significant with DN group (P-value <0.05) shown in Table 1.

One way ANOVA test was used. Data were demonstrated as mean ± SD. Statistically significant P value is < 0.05,

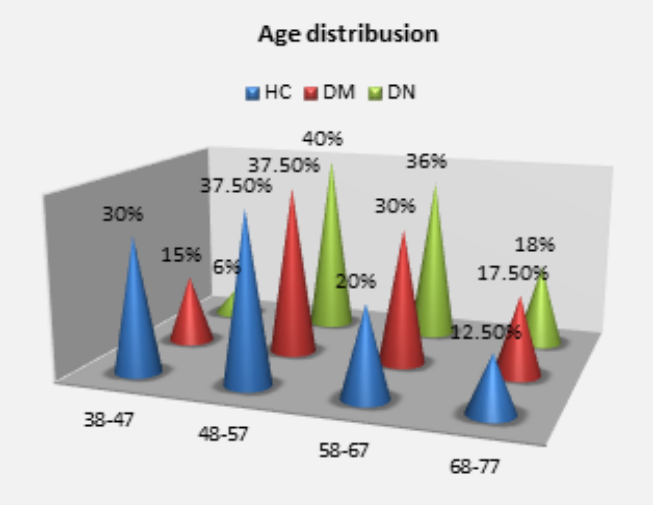

Figure 2. **Subjects distribution according to the age groups.**

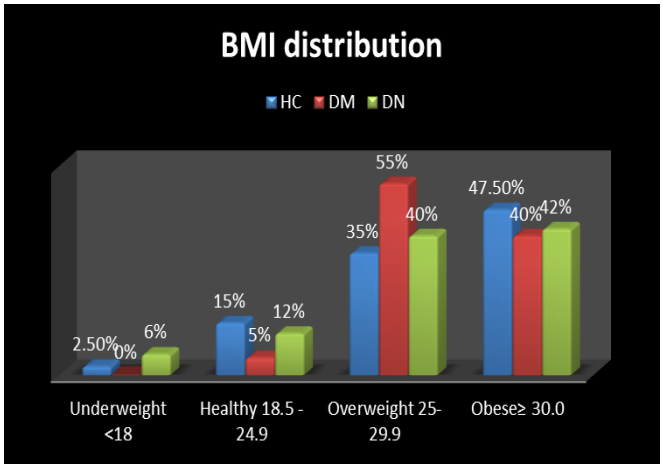

Figure 3. **BMI distribution of study individuals**.

Table 1. **Biochemical characteristics of study subjects**

Parameters	Control Mean ±SD N=40	DM Mean ±SD N=40	DN Mean ±SD N=50
Urea(mg/dL)	33.9±6.38	31.26±11.66^{c*}	149.4±42.18^{b*}
Creatinine(mg/dL)	0.67±0.13	0.8±0.18^{c*}	7.87±1.77^{b*}
eGFR(L/ min/1.73m2)	162.77±33.9	102.5±23.22^{a,c*}	7.38±1.9^{b*}
FBS(Mmol/l)	5.01±0.89	11.18±4.1^{a,c*}	14.2±5.12^{b*}
HbA1c %	5.35±0.78	9.45±1.85^{a,c*}	7.75±1.59^{b*}
TC(mg/dL)	144.8±22.83	165.35±40.36^{a,c*}	143.6±22.4
HDL(mg/dL)	47.77±9.5	38.97±13.57^{a*}	36.75±7.7^{b*}
TG(mg/dL)	112.4±30.55	184.18±55.17^{a,c*}	119.2±34.8
LDL(mg/dL)	79.3±18.77	117.68±34^{a,c*}	180.09±17.29
HOMA-IR	0.6±0.14	3.61±1.2^{a,c*}	4.5±1.7^{b*}
Insulin	78.38±23.6	64.9±22.4^{a*}	55.85±20.85^{b*}
S%	173.15±43.5	33.57±10.04^{a*}	37±12.6^{b*}
HOMA-B	74.35±20.58	67.18±17.03	53.19±13.81^{b*}
eNOS Activity	8.4±1.96	1.18±0.3^{a*}	1.05±0.32^{b*}

*a: significant difference p<0. 05 between DM and HC, *b; between DN and HC, *c: between DN and DM. Fasting blood sugar(FBS); eGFR, estimated glomerular filtration rate; total cholesterol TC; high-density lipoproteins cholesterol (HDL-C), low-density lipoproteins cholesterol (LDL-C); triglycerides(TG); Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) ; HOMA-B, used to assess pancreatic beta–cell function; S%, used to determine insulin sensitivity; glycated hemoglobin (HbA1c);, endothelial Nitric Oxide Synthase(eNOS); diabetic nephropathy(DN); diabetes mellitus (DM) and healthy control(HC).

The correlation between serum eNOS activity, creatinine and HOMA-IR in DN group was significantly negative. Similar to the correlation between serum eNOS activity, Urea and HOMA-IR in DM group as shown in Table 2.

Table 2. **Correlation between serum eNOS Activity and other clinical characteristics in HC, DM and DN patients.**

eNOS Activity(IU/min)						
Clinical Parameters	**DN(50)**		**DM(40)**		**HC(40)**	
	r	**P value**	**r**	**P value**	**r**	**P value**
BMI	- 0.1	0.4	0.6	0.07	0.35	0.1
Age(Y)	0.59	0.07	0.4	0.1	0.36	0.1
Urea (mg/dl)	- 0.1	0.38	-0.8	0.03*	- 0.04	0.79
Creatinine (mg/dl)	-0.8	0.02*	0.3	0.1	- 0.04	0.76
eGFR (L/ min/1.73m2)	- 0.05	0.6	- 0.16	0.2	- 0.05	0.71
TC(mg/dl)	0.3	0.1	- 0.08	0.5	0.9	0.06
HDL(mg/dl)	0.2	0.08	- 0.2	0.08	- 0.08	0.6
TG(mg/dl)	- 0.02	0.8	- 0.1	0.5	-0.2	0.1
LDL(mg/dl)	- 0.1	0.2	0.4	0.1	0.13	0.39
HBA1c%	- 0.05	0.7	- 0.08	0.9	0.3	0.1
FBS(Mmol/l)	0.2	0.1	- 0.11	0.48	0.1	0.37
Insulin	- 0.1	0.4	- 0.1	0.53	0.5	0.09
HOMA B	- 0.06	0.6	- 0.18	0.25	- 0.03	0.8
S%	- 0.05	0.68	0.2	0.1	0.1	0.4
HOMA_IR	-0.64	0.04*	-0.8*	0.03	0.1	0.42

r: correlation ***Significant P < 0.05, *negative correlation**

DISCUSSION

Diabetic female population in various researches is greater than male, which might be attributed to their gender characteristics. Males developed diabetic complications faster than female may be because of:

First, female appeared that their NO is more accessible which result in decrease the renal oxidative stress in comparison to the males; This could have defensive impact on development of renal impairment in diabetics.

Second, Sex hormones such as estrogens and testosterone; Estrogen tend to protect the females and hinder the advancement of non-diabetic renal disease at least before menopause .Having said that, in diabetic subjects this protection is elevated. In contrast, testosterone escalates the progression of chronic kidney disease in non-diabetic.[12]

Females with diabetes were at more risk in developing DN in comparison with males,[13] this result was consistent with other studies,[1,14] which could be explained by:

First, the equations that were used in estimating the glomerular filtration rate might be more suitable in male than female and thus overestimating disease occurrence in female.

Second, the hormonal variation between male and female, In early life and because of estrogen female tend to be more protected from renal disease; however, this defense mechanism might decline with age.

Third, social aspects have a vital role, for example, women being more frequently undergo variable screening tests with or without symptoms for reassurance or diagnosis compare to men.[15]

Forth, independent of age women tend to have more common impaired glucose tolerance (IGT) than in men.

Diabetic females were more obese than diabetic males in most studies and a stronger relationship between increased BMI and the chance of developing diabetes.[12]

In the study of Moguib included 114 females and 86 males.[1] While the study of Ali Momeni included 58 (61.7%) female and 36 (38.3%) of the patients were male.[14]

Other studies disagree with the current study where male's predominant patients for developing DN. such as the study of Albegali included 50.9% (82) males and 49.1% (79) females.[8]

After analysing the results of this study HC, DM and DN groups were age and sex matched (P- value >0.05 for both). This is identical to the study of Ali Momeni and the study of Mackawy were approved there was no significant difference based on age between patients and control groups (p>0.05).[14,16]

High BMI increased incidence of some diseases, such as diabetes mellitus, hypertension and lipid abnormalities.

Obesity as well as the group of risk factors that form the metabolic syndrome, involving hypertension, insulin resistance and dyslipidemia. These disorders are interact to produce DN and increase the severity of renal disease and finally lead to ESRD.[17]

The current study results have been showed non-significant relationship between BMI and DN, (p value >0.05) This is consist with the studies of Parineeta and Shoukry;[18,19] While disagreed with the result of [20,21].

There was a highly significant relationship among triglyceride, cholesterol, HDL and LDL and developing DN (P-value <0.001). The association of dyslipidemia and DN in this study results is clear in Table 2. This suggests a role for dyslipidemia in the advancement and progression of DN. Experimental studied have been demonstrated that lipid might produce glomerular and tubular interstitial injury [22]. This study concludes that dyslipidemia of various abnormalities is a risk factor for developing DN.

This study results agreed with the study of Jafari that shows significant relationship among TC, HDL, TG and DN (P Value <0.05) except LDL shows non-significant relationship [23]. While our study disagree with the study of Parineeta Samant which shows there is non-significant between lipid profile and DN [18].

Fasting blood sugar which is routine parameter showed significant result in Table 2 with DN (P value <0.001), the current study results agreed with the studies of [18,24].

hyperglycemia and dyslipidemia are characterization of Type 2 diabetes which connected with a collection of risk parameters forming the metabolic syndrome and leading to serious complications. Macro and micro vascular diseases resulting from High levels of glucose (and cholesterol). The high hyperglycemia (carbohydrate metabolic disorder) in diabetic patients leads to nephropathy [25].

There is significant relationship between the insulin and DN (P value <0.001) in Table 2.

In the case of insulin resistance the cells unable to utilize glucose, thus remains part of sugar in the blood; T2DM results in the non-conversion of glucose into energy, which leading to excessive amounts of sugar in the blood [26].

Blood Urea shows significant relationship with DN (p value <0.001) in Table 2, Blood Urea is an indication of renal disease .the current study results agree with the studies of [27]. While disagree with the study of Jana Makuc which reveal non- significant relationship [28].

Table 2 is also show highly significant result DN and S. Creatinine (P value < 0.001) where in presence of diabetic with duration from 9 to 12 years the S. creatinine became a hall mark of nephropathy. The current study agreed with the study of [29]. Significant relationship between DN and eGFR (P value <0.001) as in Table 2. Diabetes severity and duration strongly associated with urea levels, creatinine levels and eGFR.

Raised blood sugar causes destruction of many nephrons causing incapability of kidneys to maintain fluid and electrolyte homeostasis. Creatinine is excreted by glomerulus; hence the level of serum creatinine reflects the glomerular filtration indirectly. Decreasing the glomerular filtration rate result in raising the level of serum creatinine and urea. This increment reveals development of renal disease such as diabetic nephropathy[30].

There were significant differences in HbA1c and DN (P value <0.001) in Table 2, as in the study of [1], the current study results disagreed with the result of [19]. Uncontrolled HbA1c level is believed to be a strong indicator of uncontrolled glucose level in blood.

Significant relationship between DN and HOMA-IR (P value <0.001) in Table 2. In T2DM insulin resistance (IR) has been related to initiation and advancement of DN. The rate of occurrence of signs related to IR, like raised blood pressure, dyslipidemia and abdominal obesity also increases with stages progression of DN [31].

As regards the significant relationship between eNOS activity and DN that highlighted in this study results had agreed with the study of Amal M. Mackawy which suggested that the eNOS activity was decreased in patients with DN [16]. Impaired eNOS activity leading to endothelial dysfunction and reduced NO Production [32]. Thus, decrease in nitric oxide production leading to vascular abnormalities, such as hypertension and atherosclerosis [33] and also reduced endothelial NO generation caused gradual increase in renal disease until caused diabetic nephropathy [19,34].

CONCLUSIONS

Based on the above, we can conclude that:

As regards the age, gender and BMI among DN, DM and HC groups there was a non-significant difference.

FBS, HbA1Ic, Urea, Creatinine, eGFR and HOMA-IR considered a risk factor for developing DN.

Dyslipidemia that involves a various abnormalities considered a risk factor for developing DN.

The correlation among serum eNOS activity, serum creatinine and HOMA-IR in T2DM with DN group was significant negative.

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